PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PS3254/PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 98/00963	01/04/1998	01/04/1997
Applicant		
GLAXO GROUP LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [X] furnished by the applicant separately from the international application.
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title**,
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

Int[redacted] Application No
PCT/GB 98/00963

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 665 293 A (HAMAMATSU PHOTONICS KK) 2 August 1995 see the whole document ---	1-14, 16-20
X	WO 96 27025 A (RABANI ELY MICHAEL) 6 September 1996 see the whole document ---	1-14, 16-20
X	WO 91 06678 A (STANFORD RES INST INT ;TSIEN ROGER Y (US)) 16 May 1991 see the whole document ---	1-14, 16-20
X	WO 96 32504 A (UNIV BOSTON) 17 October 1996 see the whole document ---	15,21-24
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 July 1998	05/08/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hagenmaier, S

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No PCT/GB 98/00963

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FU D -J ET AL: "SEQUENCING DOUBLE-STRANDED DNA BY STRAND DISPLACEMENT" NUCLEIC ACIDS RESEARCH, vol. 25, no. 3, 1997, pages 677-679, XP000196977 see the whole document	15,21-24
A	WO 96 36737 A (RABANI ELY MICHAEL) 21 November 1996 see the whole document	1-24
A	DE 195 15 552 A (EUROP LAB MOLEKULARBIOLOG) 31 October 1996 see the whole document	1-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int... Application No
PCT/GB 98/00963

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0665293 A	02-08-1995	JP 7203998 A	08-08-1995
WO 9627025 A	06-09-1996	AU 5171696 A	18-09-1996
WO 9106678 A	16-05-1991	CA 2044616 A EP 0450060 A	27-04-1991 09-10-1991
WO 9632504 A	17-10-1996	AU 5544696 A EP 0830460 A	30-10-1996 25-03-1998
WO 9636737 A	21-11-1996	AU 5797696 A	29-11-1996
DE 19515552 A	31-10-1996	WO 9634114 A EP 0826067 A	31-10-1996 04-03-1998

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

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PS3254/PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 98/ 00963	01/04/1998	01/04/1997
Applicant		
GLAXO GROUP LIMITED et al.		

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   - [ ] because this figure better characterizes the invention.
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INTERNATIONAL SEARCH REPORT

Interna[redacted]l Application No
PCT[redacted] 98/00963

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 July 1998	05/08/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hagenmaier, S

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter[redacted]al Application No
PC[redacted] 98/00963

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	FU D -J ET AL: "SEQUENCING DOUBLE-STRANDED DNA BY STRAND DISPLACEMENT" NUCLEIC ACIDS RESEARCH, vol. 25, no. 3, 1997, pages 677-679, XP000196977 see the whole document ---	15,21-24
A	WO 96 36737 A (RABANI ELY MICHAEL) 21 November 1996 see the whole document ---	1-24
A	DE 195 15 552 A (EUROP LAB MOLEKULARBIOLOG) 31 October 1996 see the whole document -----	1-24

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information [redacted] patent family members

Intern[redacted]al Application No
PC[redacted] 98/00963

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0665293 A	02-08-1995	JP 7203998 A	08-08-1995
WO 9627025 A	06-09-1996	AU 5171696 A	18-09-1996
WO 9106678 A	16-05-1991	CA 2044616 A	27-04-1991
		EP 0450060 A	09-10-1991
WO 9632504 A	17-10-1996	AU 5544696 A	30-10-1996
		EP 0830460 A	25-03-1998
WO 9636737 A	21-11-1996	AU 5797696 A	29-11-1996
DE 19515552 A	31-10-1996	WO 9634114 A	31-10-1996
		EP 0826067 A	04-03-1998

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 26 October 1998 (26.10.98)	
International application No. PCT/GB98/00963	**Applicant's or agent's file reference** PS3254/PCT
International filing date (day/month/year) 01 April 1998 (01.04.98)	**Priority date** (day/month/year) 01 April 1997 (01.04.97)
Applicant KAWASHIMA, Eric, H. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 October 1998 (08.10.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Mougamadou Abidine

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PS3254/PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/00963	01/04/1998	01/04/1997
International Patent Classification (IPC) or national classification and IPC		
C12Q1/68		
Applicant		
GLAXO GROUP LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/10/1998	02.06.99
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0 Tx: 523656 epmu d Fax: (+49-89) 2399-4465	von Ballmoos, P Telephone No. (+49-89) 2399 8174

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/00963

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-46	as originally filed				

Claims, No.:

1-20,21 (part)	as originally filed				
21 (part),22-24	as received on	01/03/1999	with letter of	18/02/1999	

Drawings, sheets:

1/9-9/9	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 22, 24.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 22, 24 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-17, 23
	No:	Claims	18-21
Inventive step (IS)	Yes:	Claims	1-17
	No:	Claims	18-21, 23
Industrial applicability (IA)	Yes:	Claims	1-21, 23
	No:	Claims	---

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Part III

The subject-matter of claims 22 and 24 is unclear (Art. 6 PCT), and no examination as to novelty and inventive step was performed for the reasons set out in Part VIII.

Part V

The closest prior art appears to result from **D1 (WO 96/27025).** This document describes a method of polynucleotide sequencing (see pages 42-43). Primed DNA sample template molecules are bound to a transparent surface. Polymerization directed by each involved DNA sample template molecule is effected with an appropriate DNA polymerase and only one biotin labeled nucleotide type present during each polymerization sub-cycle step. Consequently this biotin labeled nucleotide will be added only to those sample template molecules having the base complementary to the nucleotide of said sub-cycle at the appropriate position. Nucleotides that have not been used in primer extension are washed away. After reaction with streptavidin coated beads, detection of those polynucleotides which have incorporated the label and quantification of the number of incorporated labeled nucleotides takes place by use of a video microscope. After detection, the biotin-streptavidin-bead complexes are removed and the same subcycle is then repeated in succession for each of the three remaining nucleotide types, to complete a full base sequencing cycle. Multiple said base sequence cycles are repeated and recorded data are then used to reconstruct sequence information for a segment of each sample template molecule.

It is pointed out in D1 that single molecules and not populations of molecules are used (see p. 7, l. 35-41). However, the use of single molecules leads to constraints on the detection device (see p. 13, 2. paragraph) and to detection problems if different molecules are in close proximity.

a) Claims 1-17

The examiner agrees with the applicant's discussion of D1 concerning claims 1-17. By using a plurality of molecules with the same sequences as one another at given locations, intense signals can be obtained from a small area and therefore the subject-

matter of independent claims 1 and 17 overcomes the above problems.

Since none of the available documents discloses or suggests the simultaneous step-by-step sequencing of two different populations of nucleic acid molecules the subject-matter of claims 1 and 17 appears to be novel and inventive (Art. 33(2) and 33(3) PCT).

Claims 2-16 contain preferred embodiments of the novel and inventive idea embodied in claim 1 and would also appear to meet the requirements of Articles 33(2) and 33(3) PCT.

b) Claims 18-20

Independent claim 18 relates to an apparatus, which comprises a plurality of nucleotides, a nucleic acid polymerase and detection means which can distinguish between different locations. No structural features of the apparatus are given which could define the ability to distinguish between different locations. It appears therefore that a prior art apparatus comprising a plurality of nucleotides, a nucleic acid polymerase and detection means would also be able to distinguish between different locations as long as this ability is not explicitly excluded.

D1 (WO96/27025) discloses a device (p. 43, l. 16-35; p. 12, l. 37-p. 13, l. 32) for performing a sequencing method, which uses a plurality of nucleotides (p. 42, l. 18-23), a nucleic acid polymerase (p. 42, l. 20) and which device comprises detection means. These detection means appear to be suited to distinguish between different locations (p. 13, l. 13-32).

There are no structural features in claim 18 which could distinguish the claimed apparatus from the devices disclosed in D1. Therefore claim 18 is not novel (Art. 33(2) PCT).

Dependent claims 19 and 20 are not novel either (Art. 33(2) PCT), since the removing of excess nucleotides and the repetition of reaction and detection cycles are explicitly disclosed in D1 (p. 42, l. 23-25; and p. 43, l. 10-12), and it is consequently inherent that the devices of D1 comprise means for performing these steps.

c) Claims 21 and 23

Claim 21 relates to the sequencing of a single nucleic acid and is therefore not distinguished from the method disclosed in D1. Hence, this claim is not novel (Art. 33(2) PCT).
Dependent claim 23 does not appear to involve an inventive step (Art. 33(3) PCT). The use of nick translation in sequencing methods is well known in the art (see **D2=WO 96/32504**, p. 14, l. 13-p. 15, l.9 and **D3=Nucl. Acids Research, 1997, 25(3)**, whole document) and does not appear to have any unexpected effect when applied in the present invention instead of primer extension. The skilled person, when looking for an alternative to primer extension, would therefore have selected nick translation without exercise of inventive skill, thereby arriving at the subject-matter of claim 23.

The applicant argues that claim 21 is distinguished from D1 in that the labels of the nucleotides are not washed away between the extension cycles. The examiner agrees that this feature is clearly disclosed in the description of the present application. It is also agreed that all examples in D1 include removing or neutralising of the incorporated label before the next strand extension cycle is started. The method of D1 even includes an optional further step of checking that the removal or neutralization of the label was succesful (see p. 7).
However, the wording "measuring incremental label" in present claim 21 does not unambiguously exclude the possibility of a washing step. If the applicant would have explicitly mentioned in claim 21 that no washing step takes place between steps (c) and (d), the following situation would have arisen: The skilled person would have considered from D1 that, if the label were not removed, it would interfere with the detection of incorporation of subsequent labelled nucleotides and he would not have had an indication to leave out the removal step. The possibility of measuring the changes in the cumulative signal found in the present application, which advantageously leads to a faster sequencing method, would hence have been an unexpected result.
Claim 21 and dependent claim 23 would therefore have been considered to meet the requirements of Art. 33(2) and (3) PCT.

Part VII

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 is not mentioned in the description, nor is this document identified therein.

Part VIII

a) It appears that independent claim 17 includes all the features of independent claim 1 and additionally in step c) the feature that a given nucleotide can be present in labelled and unlabelled form. In order to comply with Art. 6 and Rule 6.4 PCT, this claim should therefore have been made dependent on claim 1. This is confirmed by claim 22 which explicitly relates to this dependency.

b) The examiner agrees that claims 1-14, 16, 17 and 21 on which claim 22 is dependent, are all allowable. However, present claim 22 does itself not include any technical features. It is not unambiguously clear which technical features are encompassed by the combination of claims which is defined in claim 22, especially in view of the fact that claim 22 implies a **dependency of claim 21**, which is drafted as **independent claim**, on claims 1-14, 16 and 17. Claim 22 is therefore unclear and not allowable in its present form (Art. 6 PCT).

c) Claim 24 is unclear because its subject-matter is not defined in terms of technical features, contrary to Art. 6 and Rule 6.3 PCT.

d) Claim 23 should have been reformulated as an independent claim as it does not include **all technical features** of claims 21 and 22 but states that **instead** of hybridization a nick translation takes place (Art. 6, R. 6.4 PCT).

(c) measuring first label incorporated into the primer to determine whether, and if so, by how many base increments, the primer has been extended by incorporated of the nucleotide type;

(d) incubating the hybridized primer/target nucleic acid with a different type of nucleotide bearing a label under conditions supporting template-directed extension of the primer if the different nucleotide type can be incorporated so as to be complementary to a corresponding nucleotide in the target;

(e) measuring incremental label incorporated into the primer due to the previous incubating step to determine whether, and if so, by how many base increments, the primer has been extended by incorporation of the different nucleotide type; and

(f) repeating steps (b) – (e) until a desired portion of the target sequence can be determined from the incremental base additions to the primer.

22. A method according to claim 21 which is a method according to any of claims 1 to 14, 16 or 17.

23. A method according to claim 21 or claim 22 with the exception that instead of hybridizing a target nucleic acid molecule to a primer and extending the primer with labelled nucleotides, a nick is introduced into a double-stranded nucleic acid molecule and the nick is extended using nick translation and labelled nucleotides.

24. The invention substantially as hereinbefore described.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PS3254/PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/00963	01/04/1998	01/04/1997
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant GLAXO GROUP LIMITED et al.		

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   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/10/1998	02.06.99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. (+49-89) 2399-0 Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer von Ballmoos, P Telephone No. (+49-89) 2399 8174

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/00963

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

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1-46	as originally filed			

Claims, No.:

1-20,21 (part)	as originally filed			
21 (part),22-24	as received on	01/03/1999	with letter of	18/02/1999

Drawings, sheets:

1/9-9/9	as originally filed

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☐ the claims, Nos.:
☐ the drawings, sheets:

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☐ the entire international application.

☒ claims Nos. 22, 24.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 22, 24 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-17, 23
	No:	Claims	18-21
Inventive step (IS)	Yes:	Claims	1-17
	No:	Claims	18-21, 23
Industrial applicability (IA)	Yes:	Claims	1-21, 23
	No:	Claims	---

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Part III

The subject-matter of claims 22 and 24 is unclear (Art. 6 PCT), and no examination as to novelty and inventive step was performed for the reasons set out in Part VIII.

Part V

The closest prior art appears to result from **D1 (WO 96/27025).** This document describes a method of polynucleotide sequencing (see pages 42-43). Primed DNA sample template molecules are bound to a transparent surface. Polymerization directed by each involved DNA sample template molecule is effected with an appropriate DNA polymerase and only one biotin labeled nucleotide type present during each polymerization sub-cycle step. Consequently this biotin labeled nucleotide will be added only to those sample template molecules having the base complementary to the nucleotide of said sub-cycle at the appropriate position. Nucleotides that have not been used in primer extension are washed away. After reaction with streptavidin coated beads, detection of those polynucleotides which have incorporated the label and quantification of the number of incorporated labeled nucleotides takes place by use of a video microscope. After detection, the biotin-streptavidin-bead complexes are removed and the same subcycle is then repeated in succession for each of the three remaining nucleotide types, to complete a full base sequencing cycle. Multiple said base sequence cycles are repeated and recorded data are then used to reconstruct sequence information for a segment of each sample template molecule.

It is pointed out in D1 that single molecules and not populations of molecules are used (see p. 7, l. 35-41). However, the use of single molecules leads to constraints on the detection device (see p. 13, 2. paragraph) and to detection problems if different molecules are in close proximity.

a) Claims 1-17

The examiner agrees with the applicant's discussion of D1 concerning claims 1-17. By using a plurality of molecules with the same sequences as one another at given locations, intense signals can be obtained from a small area and therefore the subject-

matter of independent claims 1 and 17 overcomes the above problems.

Since none of the available documents discloses or suggests the simultaneous step-by-step sequencing of two different populations of nucleic acid molecules the subject-matter of claims 1 and 17 appears to be novel and inventive (Art. 33(2) and 33(3) PCT).

Claims 2-16 contain preferred embodiments of the novel and inventive idea embodied in claim 1 and would also appear to meet the requirements of Articles 33(2) and 33(3) PCT.

b) Claims 18-20

Independent claim 18 relates to an apparatus, which comprises a plurality of nucleotides, a nucleic acid polymerase and detection means which can distinguish between different locations. No structural features of the apparatus are given which could define the ability to distinguish between different locations. It appears therefore that a prior art apparatus comprising a plurality of nucleotides, a nucleic acid polymerase and detection means would also be able to distinguish between different locations as long as this ability is not explicitly excluded.

D1 (WO96/27025) discloses a device (p. 43, l. 16-35; p. 12, l. 37-p. 13, l. 32) for performing a sequencing method, which uses a plurality of nucleotides (p. 42, l. 18-23), a nucleic acid polymerase (p. 42, l. 20) and which device comprises detection means. These detection means appear to be suited to distinguish between different locations (p. 13, l. 13-32).

There are no structural features in claim 18 which could distinguish the claimed apparatus from the devices disclosed in D1. Therefore claim 18 is not novel (Art. 33(2) PCT).

Dependent claims 19 and 20 are not novel either (Art. 33(2) PCT), since the removing of excess nucleotides and the repetition of reaction and detection cycles are explicitly disclosed in D1 (p. 42, l. 23-25; and p. 43, l. 10-12), and it is consequently inherent that the devices of D1 comprise means for performing these steps.

c) Claims 21 and 23

Claim 21 relates to the sequencing of a single nucleic acid and is therefore not distinguished from the method disclosed in D1. Hence, this claim is not novel (Art. 33(2) PCT).
Dependent claim 23 does not appear to involve an inventive step (Art. 33(3) PCT). The use of nick translation in sequencing methods is well known in the art (see **D2=WO 96/32504**, p. 14, l. 13-p. 15, l.9 and **D3=Nucl. Acids Research, 1997, 25(3)**, whole document) and does not appear to have any unexpected effect when applied in the present invention instead of primer extension. The skilled person, when looking for an alternative to primer extension, would therefore have selected nick translation without exercise of inventive skill, thereby arriving at the subject-matter of claim 23.

The applicant argues that claim 21 is distinguished from D1 in that the labels of the nucleotides are not washed away between the extension cycles. The examiner agrees that this feature is clearly disclosed in the description of the present application. It is also agreed that all examples in D1 include removing or neutralising of the incorporated label before the next strand extension cycle is started. The method of D1 even includes an optional further step of checking that the removal or neutralization of the label was succesful (see p. 7).
However, the wording "measuring incremental label" in present claim 21 does not unambiguously exclude the possibility of a washing step. If the applicant would have explicitly mentioned in claim 21 that no washing step takes place between steps (c) and (d), the following situation would have arisen: The skilled person would have considered from D1 that, if the label were not removed, it would interfere with the detection of incorporation of subsequent labelled nucleotides and he would not have had an indication to leave out the removal step. The possibility of measuring the changes in the cumulative signal found in the present application, which advantageously leads to a faster sequencing method, would hence have been an unexpected result.
Claim 21 and dependent claim 23 would therefore have been considered to meet the requirements of Art. 33(2) and (3) PCT.

Part VII

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 is not mentioned in the description, nor is this document identified therein.

Part VIII

a) It appears that independent claim 17 includes all the features of independent claim 1 and additionally in step c) the feature that a given nucleotide can be present in labelled and unlabelled form. In order to comply with Art. 6 and Rule 6.4 PCT, this claim should therefore have been made dependent on claim 1. This is confirmed by claim 22 which explicitly relates to this dependency.

b) The examiner agrees that claims 1-14, 16, 17 and 21 on which claim 22 is dependent, are all allowable. However, present claim 22 does itself not include any technical features. It is not unambiguously clear which technical features are encompassed by the combination of claims which is defined in claim 22, especially in view of the fact that claim 22 implies a **dependency of claim 21**, which is drafted as **independent claim**, on claims 1-14, 16 and 17. Claim 22 is therefore unclear and not allowable in its present form (Art. 6 PCT).

c) Claim 24 is unclear because its subject-matter is not defined in terms of technical features, contrary to Art. 6 and Rule 6.3 PCT.

d) Claim 23 should have been reformulated as an independent claim as it does not include **all technical features** of claims 21 and 22 but states that **instead** of hybridization a nick translation takes place (Art. 6, R. 6.4 PCT).

(c) measuring first label incorporated into the primer to determine whether, and if so, by how many base increments, the primer has been extended by incorporated of the nucleotide type;

(d) incubating the hybridized primer/target nucleic acid with a different type of nucleotide bearing a label under conditions supporting template-directed extension of the primer if the different nucleotide type can be incorporated so as to be complementary to a corresponding nucleotide in the target;

(e) measuring incremental label incorporated into the primer due to the previous incubating step to determine whether, and if so, by how many base increments, the primer has been extended by incorporation of the different nucleotide type; and

(f) repeating steps (b) – (e) until a desired portion of the target sequence can be determined from the incremental base additions to the primer.

22. A method according to claim 21 which is a method according to any of claims 1 to 14, 16 or 17.

23. A method according to claim 21 or claim 22 with the exception that instead of hybridizing a target nucleic acid molecule to a primer and extending the primer with labelled nucleotides, a nick is introduced into a double-stranded nucleic acid molecule and the nick is extended using nick translation and labelled nucleotides.

24. The invention substantially as hereinbefore described.